PATENT SPECIFICATION

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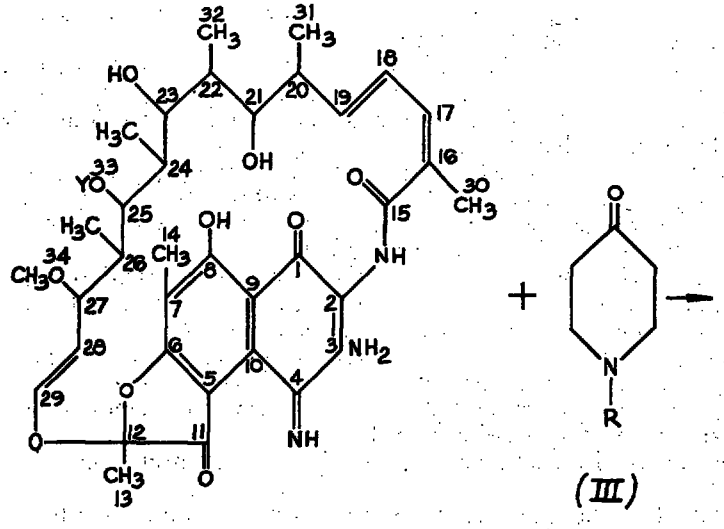


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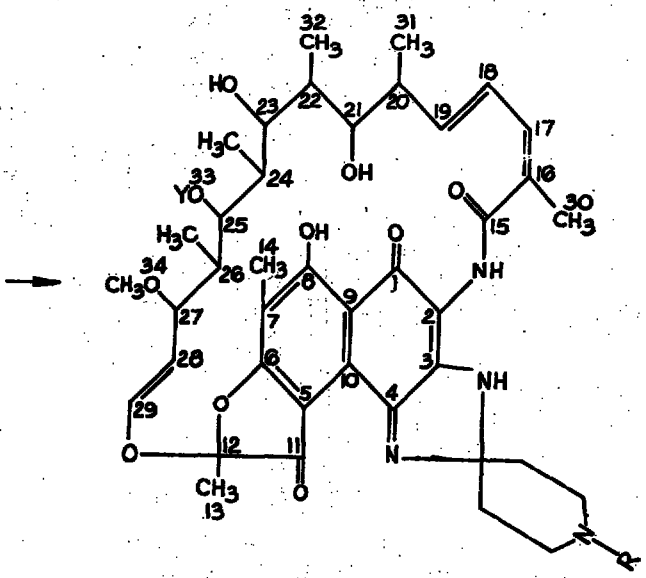
(54) RIFAMYCIN COMPOUNDS

(71) We, ARCHIFAR LABORATORI CHIMICO FARMACOLOGICI S.p.A., an Italin Joint Stock Company, of C. so Verona 165, Rovereto, Italy, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

This invention relates to novel rifamycin compounds, to processes for their preparation and to pharmaceutical compositions and preparations containing them, and is an improvement in, or modification of, the invention of our Patent No. 1,542,063. The rifamycin compounds according to the above mentioned patent have antibacterial activity *in vitro* on Gram-positive, Gram-negative and particularly on *Mycobacterium tuberculosis*. According to the present invention it has been shown that rifamycin compounds obtained by reacting 3 - amino - 4 - desoxo - 4 - iminorifamycin S with certain 4-piperidones having the following structural formula III



SPECIFICATION AMENDED - SEE ATTACHED SLIP



(I)

have good therapeutic activity as assessed by *in vitro* tests on mice infected by intravenous application of *Mycobacterium tubercolis*.

In one aspect the present invention provides novel rifamycin compounds of formula I where R is selected from hydrogen, linear C_4—C_8 alkyl, branched C_3—C_8 alkyl, C_3—C_4 alkenyl, C_3—C_8 cycloalkyl, C_7—C_8 cycloalkyl-alkyl, C_3—C_7 alkoxyalkyl, C_5—C_8 alkyl-furyl, C_5—C_8 alkyl-tetrahydrofuryl, C_5—C_8 alkanoyl and C_2—C_8 monohaloalkanoyl; and Y is —H or —$COCH_3$.

Where R is a branched alkyl radical, it can be, for example, one having 4, 5 or 6 carbon atoms. In other compounds according to said one aspect of the invention, R can be a 2-alkyl-furyl radical or a 2-alkyl-tetrahydrofuryl radical.

Compounds of the present invention have been found by us to have good activity in *in vivo* tests on white mice CD1.

Among the piperidinylidene derivatives claimed in Patent No. 1,542,063, the rifamycin compounds of formula I, obtained by reacting a rifamycin compound of formula II with N-ethyl-4-piperidone and hereinafter called "ethyl derivative", proved to have the highest therapeutic activity.

The rifamycin compounds of the present invention have been found to have therapeutic activity surprisingly higher than that of rifamycin compounds obtained by using 4-piperidones disclosed in the above mentioned patent. This higher activity is shown by the following experiments.

White mice CD1 were infected with *Mycobacterium tuberculosis* $H_{37}RV$ culture by intravenous application, 0.2 ml of the above mentioned culture, containing twice the LD_{50} amount, being injected into each mouse.

Three days after the mice were infected, they were treated by oral application with rifamycin compounds of the invention at doses ranging from 20 to 1.25 mg/Kg. Groups of fifteen mice were treated with each rifamycin compound in single daily doses, for five days in succession and during six weeks.

At the end of the treatments the deaths were registered and the PD_{50} was calculated.

The animals were kept under observation during about two months.

The "ethyl derivative" PD_{50} was 20 mg/Kg.

The compounds tested are indicated in the following table by their substituent R, radical Y in each case being hydrogen. The table also shows the results of the *in vivo* tests and of the *in vitro* tests and also the UV-visible absorption spectrum.

TABLE I
Activity

R	In vitro MIC μg/ml	In vivo PD_{50} mg/Kg	UV-visible absorption spectrum in methanol: peaks observed at –nm
n-butyl	0.0005	5	496, 317, 276, 240
n-hexyl	0.0005	10	497, 314, 278, 239
allyl	0.0012	5	491, 314, 276, 235
i-butyl	0.0012	2.6	493, 315, 274, 238
methyl-allyl	0.005	5	498, 313, 275, 238
sec-butyl	0.005	3.7	500, 315, 275, 240
2-methyl-furyl	0.0012	—	—
1,2-dimethyl-propyl	0.005	2.5	—
n-pentyl	0.0012	5	500, 316, 278, 240
1,3-dimethyl-butyl	0.0012	2.5	500, 315, 277, 240
3-pentyl	0.0012	2.5	500, 315, 276, 240
ethyl	0.01	20	—

The results obtained through these experiments with the "ethyl derivative" and with some others of the new compounds are reported in Table I and demonstrate that the new derivatives have a PD_{50} surprisingly lower than the "ethyl derivative" and therefore are more effective than the ethyl derivative.

The rifamycin compounds of the invention can be obtained by a process (which constitutes a second aspect of the invention) in which a compound of formula II as herein depicted and in which Y is —H or —$COCH_3$ is reacted with a 4-piperidone of formula III as herein depicted and in which R is hydrogen, a straight-chain alkyl radical having 4 to 8 carbon atoms, a branched-chain alkyl radical having 3 to 8 carbon atoms, an alkenyl radical having 3 or 4 carbon atoms, a cycloalkyl radical having 3 to 6 carbon atoms a cycloalkylalkyl radical having 7 or 8 carbon atoms, an alkoxyalkyl radical having 3 to 7 carbon atoms, an alkyl-furyl radical having 5 or 6 carbon atoms, an alkyl-tetrahydrofuryl radical having 5 or 6 carbon atoms, an alkanoyl radical having 5 or 6 carbon atoms or a monohaloalkanoyl radical having 2 to 6 carbon atoms.

The process is conveniently carried out in the presence of zinc and acetic acid or zinc and ammonium acetate.

A third aspect of the invention comprises pharmaceutical compositions which comprise a rifamycin compound of the invention and a pharmaceutically-acceptable carrier or diluent therefor.

A fourth aspect of the invention comprises pharmaceutical preparations which comprise a rifamycin compound or pharmaceutical composition of the invention, in unit dosage form.

The invention is illustrated by the following Examples.

EXAMPLE 1

8 g 3 - amino - 4 - deoxo - 4 - imino - rifamycin S were dissolved in 40 ml dichloromethane and reacted with 2.6 g 1 - n - hexyl - 4 - piperidone at +5°C for 48 hours. The solution was diluted with 600 ml ethyl ether, filtered and washed with water. The organic phase was dried on sodium sulphate and then evaporated to dryness. The residue was extracted with ligroin and the violet solution evaporated to dryness. Yield: 2.5 g product of formula (I), wherein Y is —$COCH_3$, and R is a n-hexyl radical. The electronic absorption spectrum in methanol shows peaks at 497, 314, 278 and 239 nm.

EXAMPLE 2

8 g 3 - amino - 4 - deoxo - 4 - imino - rifamycin S were dissolved in 40 ml tetrahydrofuran. 4 g 1 - (1',3' - dimethyl - butyl) - 4 - piperidone, 0.5 g zinc and 0.5 g ammonium acetate were added and the mixture was stirred at room temperature for 30 minutes. The reaction mixture was worked up as in the Example No. 1 obtaining 3.5 g of a product of formula (I), wherein Y is —$COCH_3$ and R is a 1,3-dimethyl-butyl radical. The electronic absorption spectrum in methanol shows peaks at 500, 315, 277 and 240 nm.

EXAMPLE 3

8 g 3 - amino - 4 - deoxo - 4 - imino - rifamycin S were dissolved in 40 ml tetrahydrofuran . 1.8 g 1-methallyl-4-piperidone, 0.2 g zinc and 0.2 g ammonium acetate were added and the mixture was allowed to stand at +5°C for one night.

Reaction mixture was worked up as in the Example No. 1 obtaining 5.5 g product of formula (I), wherein Y is —$COCH_3$, and R is a methallyl radical.

The electronic absorption spectrum in methanol shows peaks at 498, 313, 275 and 238 nm.

EXAMPLE 4

8 g 3 - amino - 4 - deoxo - 4 - imino - rifamycin S were dissolved in 40 ml tetrahydrofuran. 3 g 1-cyclohexyl-4-piperidone, 0.2 g zinc and 0.2 ammonium acetate were added and the mixture was stirred for 2.5 hours at room temperature. Unreacted zinc was filtered off and the solution diluted with 1000 ml ethyl ether. The ethereal solution was washed with sodium phosphate buffer solution at pH 7.8 and then extracted with diluted acetic acid. The violet aqueous solution was extracted with chloroform, the organic phase was washed with water and then dried on sodium sulfate. The chloroform was evaporated to dryness. Yield: 3.8 g product of formula (I), wherein Y is —$COCH_3$, and R is a cyclohexyl radical. The electronic absorption spectrum in methanol shows peaks at 498, 312, 273 and 235 nm.

EXAMPLE 5

8 g 3 - amino - 4 - deoxo - 4 - imino - rifamycin S were dissolved in 40 ml tetrahydrofuran. 0.5 g zinc, 0.5 g ammonium acetate and 5.5 g 1 - 2' - methylfuryl - 4 - piperidone were added and the mixure was stirred at room temperature for 24 hours. The reaction mixture was filtered, diluted with 500 ml diethyl ether and washed with water. The diluted organic phase was concentrated at 250 ml and then extracted with aqueous acetic acid. The violet, aqueous solution was extracted with dichloromethane and the organic phase, washed with water and dried on sodium sulfate, was evaporated to dryness. Yield: 3.3 g product of formula (I) wherein Y is —$COCH_3$ and R is a 2'-methylfuryl radical. The electronic absorption spectrum in methanol shows peaks at 497, 316, 276 and 240 nm.

EXAMPLE 6

8 g 3 - amino - 4 - deoxo - 4 - imino - rifamycin S were dissolved in 40 ml tetrahydrofuran and dropped at 50°C into a mixture of 15 ml tetrahydrofuran, 5 ml acetic acid, 1 g zinc and 5 g 1 - (2' - methyltetrahydrofuryl) - 4 - piperidone. Heating is continued for 30 minutes and then the reaction mixture was worked up as in the Example No. 5. Yield: 2.1 g product of formula (I) wherein Y is —$COCH_3$ and R is a 2'-methyl-tetrahydrofuryl radical. The electronic absorption spectrum in methanol shows peaks at 495, 314, 275 and 239 nm.

EXAMPLE 7

32 g 3 - amino - 4 - deoxo - 4 - imino - rifamycin S were dissolved in 200 ml tetrahydrofuran. 9 g 4-piperidone monohydrate hydrochloride, 10 g ammonium acetate and 0.4 g zinc were added and the mixture was stirred at room temperature for 12 hours. The reaction mixture was filtered and dropped into 1500 ml diluted acetic acid. After filtration the aqueous solution was neutralized with sodium bicarbonate at pH 6 and then extracted twice with dichloromethane. Yield: 13.4 g product of formula (I), wherein Y is —$COCH_3$ and R is hydrogen. The electronic absorption spectrum in methanol shows peaks at 500, 315, 275 and 240 nm.

EXAMPLE 8

8 g 3 - amino - 4 - deoxo - 4 - imino - rifamycin S were dissolved in 50 ml tetrahydrofuran. 0.3 g zinc, 0.3 g ammonium acetate and 2.5 g 1-chloroacetyl-4-piperidone were added and the mixture allowed to react at +5°C for 48 hours. The reaction mixture was filtered and diluted with 150 ml dichloromethane and 800 ml cyclohexane. The solution was filtered again, washed with sodium phosphate buffer solution at pH 7.5 and then with water. The solvent was evaporated under vacuum and the residue was crystallized from cyclohexane. Yield: 3.2 g product of formula (I), wherein Y is —$COCH_3$, and R is a chloroacetyl radical. The electronic absorption spectrum in methanol shows peaks at 497, 310, 273 and 235 nm.

EXAMPLE 9

8 g 3 - amino - 4 - deoxo - 4 - imino - rifamycin S were dissolved in 40 ml tetrahydrofuran. 0.5 g zinc, 5 ml acetic acid and 4.5 g 1-n-octyl-4-piperidone were

added and the mixture was stirred for ten minutes at room temperature. Unreacted zinc was filtered off and the solution diluted with 700 ml diisopropyl ether. The solution was filtered again and concentrated to 300 ml under vacuum. 300 ml petroleum ether were added and the solution was filtered once more. After evaporation of the solvent the oily residue was dissolved in 40 ml methanol and the solution was dropped in 400 ml water. The obtained precipitate was filtered off, washed with water and dried at 40°C under vacuum. Yield: 3.8 g product of formula (I), wherein Y is —$COCH_3$ and R is a n-octyl radical. The electronic absorption spectrum in methanol shows peaks at 497, 310, 274 and 236 nm.

EXAMPLE 10

16 g 3 - amino - 4 - deoxo - 4 - imino - rifamycin S were dissolved in 100 ml tetrahydrofuran. 1 g zinc, 0.5 g ammonium acetate and 8 g 1 - (3' - methoxy - propyl) - 4 - piperidone were added and the mixture was stirred at room temperature for 60 minutes. The reaction mixture was filtered, diluted with 1500 ml xylene and washed with water. The organic phase was extracted with diluted acetic acid and then discharged. The aqueous solution, buffered at pH 7 with sodium phosphate solution, was extracted with dichloromethane. After dilution with petroleum ether the violet solution was filtered and then evaporated to dryness. Yield: 3.0 g product of formula (I), wherein Y is —$COCH_3$, and R is a 3-methoxy-propyl radical. Thin layer chromatography on silica gel plates, using chloroform-methanol 9:1 as mobile phase, showed one violet spot with Rf=0.48.

EXAMPLE 11

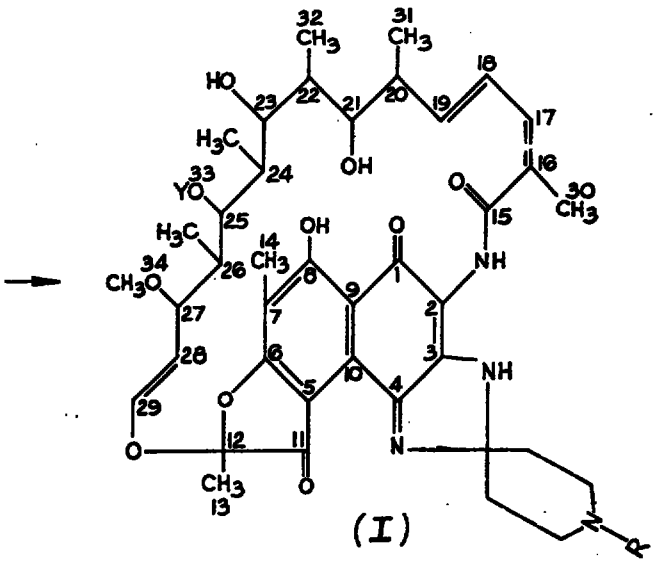
8 g 3 - amino - 4 - deoxo - 4 - imino - rifamycin S were dissolved in 40 ml tetrahydrofuran. 0.5 g zinc, 0.5 g ammonium acetate and 4.5 g 1 - (1',4' - dimethyl - pentyl) - 4 - piperidone were added and the mixture was stirred at room temperature for 30 minutes. The reaction mixture was worked up as in the Example No. 10. Yield: 5.0 g product of formula (I) wherein Y is —$COCH_3$ and R is a 1,4-dimethylpentyl radical. Thin layer chromatography on silica gel plates, using chloroform-methanol 9:1 as mobile phase, showed one violet spot with Rf=0.52.

EXAMPLE 12

8 g 3 - amino - 4 - deoxo - 4 - imino - rifamycin S were dissolved in 50 ml tetrahydrofuran. 0.2 g zinc, 0.2 g ammonium acetate and 3 g 1-pivaloyl-4-piperidone were added and the mixture was kept at 0°C for 3 days. The reaction mixture was filtered, diluted with 300 ml diethyl ether and washed with sodium phosphate buffer solution at pH 7.5. The organic phase was washed with water, dried on sodium sulfate and evaporated to dryness. The residue was crystallized from cyclohexane. Yield: 7 g product of formula (I) wherein Y is —$COCH_3$ and R is a pivaloyl radical. The electronic absorption spectrum in methanol shows peaks at 497, 316, 276 and 238 nm.

WHAT WE CLAIM IS:—

1. Rifamycin compounds having formula



(I)

added and the mixture was stirred for ten minutes at room temperature. Unreacted zinc was filtered off and the solution diluted with 700 ml diisopropyl ether. The solution was filtered again and concentrated to 300 ml under vacuum. 300 ml petroleum ether were added and the solution was filtered once more. After evaporation of the solvent the oily residue was dissolved in 40 ml methanol and the solution was dropped in 400 ml water. The obtained precipitate was filtered off, washed with water and dried at 40°C under vacuum. Yield: 3.8 g product of formula (I), wherein Y is —$COCH_3$ and R is a n-octyl radical. The electronic absorption spectrum in methanol shows peaks at 497, 310, 274 and 236 nm.

EXAMPLE 10

16 g 3 - amino - 4 - deoxo - 4 - imino - rifamycin S were dissolved in 100 ml tetrahydrofuran. 1 g zinc, 0.5 g ammonium acetate and 8 g 1 - (3' - methoxy - propyl) - 4 - piperidone were added and the mixture was stirred at room temperature for 60 minutes. The reaction mixture was filtered, diluted with 1500 ml xylene and washed with water. The organic phase was extracted with diluted acetic acid and then discharged. The aqueous solution, buffered at pH 7 with sodium phosphate solution, was extracted with dichloromethane. After dilution with petroleum ether the violet solution was filtered and then evaporated to dryness. Yield: 3.0 g product of formula (I), wherein Y is —$COCH_3$, and R is a 3-methoxy-propyl radical. Thin layer chromatography on silica gel plates, using chloroform-methanol 9:1 as mobile phase, showed one violet spot with Rf=0.48.

EXAMPLE 11

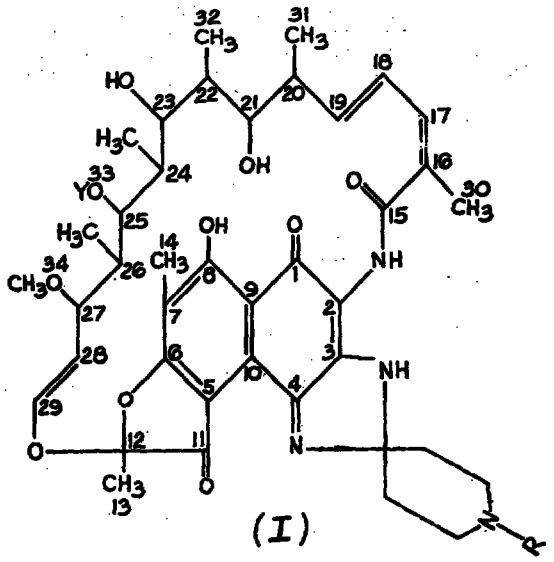
8 g 3 - amino - 4 - deoxo - 4 - imino - rifamycin S were dissolved in 40 ml tetrahydrofuran. 0.5 g zinc, 0.5 g ammonium acetate and 4.5 g 1 - (1',4' - dimethyl - pentyl) - 4 - piperidone were added and the mixture was stirred at room temperature for 30 minutes. The reaction mixture was worked up as in the Example No. 10. Yield: 5.0 g product of formula (I) wherein Y is —$COCH_3$ and R is a 1,4-dimethylpentyl radical. Thin layer chromatography on silica gel plates, using chloroform-methanol 9:1 as mobile phase, showed one violet spot with Rf=0.52.

EXAMPLE 12

8 g 3 - amino - 4 - deoxo - 4 - imino - rifamycin S were dissolved in 50 ml tetrahydrofuran. 0.2 g zinc, 0.2 g ammonium acetate and 3 g 1-pivaloyl-4-piperidone were added and the mixture was kept at 0°C for 3 days. The reaction mixture was filtered, diluted with 300 ml diethyl ether and washed with sodium phosphate buffer solution at pH 7.5. The organic phase was washed with water, dried on sodium sulfate and evaporated to dryness. The residue was crystallized from cyclohexane. Yield: 7 g product of formula (I) wherein Y is —$COCH_3$ and R is a pivaloyl radical. The electronic absorption spectrum in methanol shows peaks at 497, 316, 276 and 238 nm.

WHAT WE CLAIM IS:—

1. Rifamycin compounds having formula



(I)

wherein R is selected from hydrogen, linear C_4—C_8 alkyl, branched C_3—C_8 alkyl, C_3—C_4 alkenyl, C_3—C_8 cycloalkyl, C_7—C_8 cycloalkyl-alkyl, C_3—C_7 alkoxyalkyl, C_5—C_8 alkyl-furyl, C_5—C_8 alkyl-tetrahydrofuryl, C_5—C_6 alkanoyl and C_2—C_6 monohaloalkanoyl, and Y is —H or —$COCH_3$.

2. A compound according to Claim 1, in which R is a branched alkyl radical having 4, 5 or 6 carbon atoms.

3. A compound according to Claim 1, in which R is a 2-alkyl-furyl radical or a 2-alkyl-tetrahydrofuryl radical.

4. A compound according to Claim 3, in which R is a 2-alkyl-tetrahydrofuryl radical.

5. A compound according to Claim 2, 3 or 4 in which Y is —$COCH_3$.

6. A rifamycin compound obtained as the product of any of the Examples.

7. A process for preparing a rifamycin compound as claimed in any of Claims 1 to 6, in which a compound of formula (II) as herein depicted and in which Y is —H or —$COCH_3$ is reacted with a 4-piperidone of formula (III) as herein depicted and in which R is hydrogen, a straight-chain alkyl radical having 4 to 8 carbon atoms, a branched-chain alkyl radical having 3 to 8 carbon atoms, an alkenyl radical having 3 or 4 carbon atoms, a cycloalkyl radical having 3 to 6 carbon atoms, a cycloalkylalkyl radical having 7 or 8 carbon atoms, an alkoxyalkyl radical having 3 to 7 carbon atoms, an alkyl-furyl radical having 5 or 6 carbon atoms, an alkyl-tetrahydrofuryl radical having 5 or 6 carbon atoms, an alkanoyl radical having 5 or 6 carbon atoms or a monohaloalkanoyl radical having 2 to 6 carbon atoms.

8. A process according to Claim 7 in which said reaction with the piperidone is carried out in the presence of zinc and acetic acid.

9. A process according to Claim 7 in which said reaction with the piperidone is carried out in the presence of zinc and ammonium acetate.

10. A process according to Claim 7, substantially as described herein.

11. A process for preparing a rifamycin compound of Formula (I) herein where R and Y are as defined in Claim 1, substantially as described in any of the Examples.

12. A rifamycin compound when obtained by the process of any of Claims 7 to 11.

13. A pharmaceutical composition which comprises a rifamycin compound as claimed in any of Claims 1 to 6 and 12 and a pharmaceutically-acceptable carrier or diluent therefor.

14. A pharmaceutical preparation which comprises a rifamycin compound as claimed in any of Claims 1 to 6 and 12, or a pharmaceutical composition of Claim 13, in unit dosage form.

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By a direction given under Section 30 of the Patents Act 1977 this application proceeded in the name of FARMITALIA CARLO ERBA S.p.A. an Italian Corporation of Via Imbonati 24, 20159 Milan, Italy.

THE PATENT OFFICE

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